ANNALES MEDICINAE EXPERIMENTALIS ET BIOLOGIAE FENNIAE

INVESTIGATIONS ON ESCHERICHIA COLI O GROUPS 1—25, 44 AND 78 AND SERO-TYPES 26:B6, 55:B5, 86:B7, 111:B4, 125:B15 AND 126:B16

OCCURRENCE IN FAECES OF HEALTHY AND DIARRHOEAL INFANTS

BY

J. A. GRÖNROOS

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MERCATORIN KIRJAPAINO
HELSINKI, FINLAND

FROM THE DEPARTMENT OF MEDICAL MICROBIOLOGY AND FROM THE CHILDREN'S CLINIC, UNIVERSITY OF TURKU

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To My Parents

CONTENTS

INTRODUCTION [1]

The various observations made prior to 1940 on the serological properties of the *Escherichia* genus were heterogeneous in such a degree that a serological classification of *Escherichia* strains was not thought possible. Despite the discovery by Smith in 1927 of a thermostabile antigen which prevented the agglutination of living capsular *Escherichia* bacilli in O immune sera prepared with acapsular forms, and although Julianelle was able to establish in 1926 the possibility of classifying coliform bacilli serologically on the basis of their capsular antigens, the observations were, however, scattered ones and their value for the serological classification of the genus *Escherichia* was not realized. During the 1940's, however, Kauffmann and his coworkers succeeded in clarifying the serological properties of the *Escherichia* bacilli and were then able to classify strains belonging to the genus *Escherichia* into clearly defined types on the basis of their antigenic structures.

In 1944 Kauffmann was able to conclude that strains belonging to certain O groups are more often encountered than strains belonging to other O groups. Kauffmann, Knipschildt and Vahlne then devised a diagnostic schema for the genus *Escherichia* in which they included only O groups to which *Escherichia* strains isolated from pathological processes had been frequently found to belong. The schema comprised groups characterized by O antigens 1—25.

A special group of *Escherichia* types is formed by those whose causal relationship to infantile diarrhoea has been postulated by numerous investigators. Such *E.coli* sero-types are 26:B6, 55:B5,

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[1] A brief report on the results of this investigation was presented at the Eleventh Scandinavian Congress of Pathology and Bacteriology at Aarhus in June, 1955 (Grönroos 1956).

86:B7, 111:B4, 112 a,c:B11, 119:B14, 124:B17, 125:B15, 126:B16, 127:B8, and 128:B12. These types are listed up to 1955 by Edwards and Ewing (1955). The O groups of the aforementioned sero-types were not included in the diagnostic antigenic schema, evidently because the schema was derived on the basis of strains from material which did originate from cases of infantile diarrhoea.

In connection with an earlier investigation (1954), the author observed that *E.coli* strains which had previously been encountered in sporadic cases of infantile diarrhoea could be isolated in only a relatively low frequency in a fairly large number of cases of infantile diarrhoea. This gave cause to investigate whether *E.coli* types of the diagnostic antigenic schema might be isolated in significant numbers from diarrhoeal infants. Since little was then known about the occurrence of *Escherichia* strains of the first 25 O groups in faeces of infants, and since strains of these groups have been considered by several authors (Kauffmann 1943, Knipschildt 1945, Vahlne 1945, Ewertsen 1946 and Sjöstedt 1946) to possess a certain degree of pathogenicity, an investigation was therefore begun in 1952 in which *the main object was to determine to what extent E.coli strains belonging to the groups included in the diagnostic antigenic schema occur in the faeces of healthy infants and infants suffering from diarrhoea.* Also the occurrence of the *E.coli* types 26:B6, 55:B5: 86:B7 and 111:B4 which have previously been found of aetiological significance in infantile diarrhoea has been studied. In addition to these types, the occurrence of certain other types, 44:74L, 125:B15 and 126:B16, which have been previously isolated from faeces of infants with diarrhoea and strains belonging to O group 78 frequently isolated from calf scours has been investigated.

Attention has also been paid to the serological identification of the encountered strains.

PREVIOUS INVESTIGATIONS

After discovering in 1943 the thermolabile L antigen which prevents O agglutination in O immune serum, Kauffmann succeeded in allocating the *Escherichia coli* bacilli to well-defined serological groups. He observed further that most strains isolated from pathological conditions could be entered into a relatively small number of O groups. The serology of *Escherichia* was further elucidated by the studies of Knipschildt and Vahlne.

Kauffmann (1943) encountered in his studies three O inagglutinable strains which could not be rendered agglutinable even by heat treatment. This property Knipschildt (1945) was able to show to be due to the presence of a more heat-resistent A antigen. Vahlne (1945) found later that the A antigen could be destroyed by heating the strains 2 ½ hrs at 120° C., whereupon they became O agglutinable. Knipschildt (1945) detected a further antigen, the B antigen, whose ability to prevent O agglutination was destroyed by heating at 100° C., but which differed from the L antigen in that it retained its ability to bind agglutinins after the heat treatment. The L, A and B antigens are usually referred to as K antigens (Kauffmann 1954). The antigenic differences as to agglutinability, agglutinin-binding capacity and agglutinogenic capacity after treatment with formalin and heat are summarized in Table 1 (Kauffmann 1954).

TABLE 1

ANTIGENIC DIFFERENCES BETWEEN VARIOUS K ANTIGENS

(According to Kauffmann and Knipschildt)

Treatment of culture		L	A	B
Living or formolized (0.5 %)	1	+	+	+
	2	+	+	+
	3	+	+	+
Heated 2 ½ hrs. at 100° C.	1	—	+	—
	2	—	+	+
	3	—	(+)	—

1 = agglutinability; 2 = agglutinin-binding capacity;
3 = agglutinogenic capacity

The diagnostic antigenic schema of *Escherichia coli* comprised 20 O groups when first published by Kauffmann (1944). Five new O groups were added to the schema on the basis of Knipschildt's investigations (1945). The schema was elaborated further by Kauffmann, Knipschildt and Vahlne who demonstrated the existence of new K and H antigens. The majority of the 25 O groups were thus found to consist of several sub-types with different K and H antigens. The relationships between the 25 O antigens were studied by these authors by means of cross-agglutination tests. The results indicated that overlapping reactions may occur between some O antigens but with a certain regularity; in most cases the overlapping reactions occurred at low titres only. Ewing et al. (1956) have tested 137 standard O group strains in each of the 137 O antisera. The interrelationships were compared with those observed by Kauffmann and coworkers and "it was found that while there were a few deviations with respect to minor relationships, the results with regard to major antigenic relationships were similar". According to the results of Vahlne (1945), overlapping reactions obviously rarely occur in K immune sera. The findings of Ewing et al. (1956) were similar. They stated that the relationships between K antigens are relatively low in titre and do not appear to be important.

Vahlne (1945) and Ewing et al. (1956) observed only some overlapping reactions among H antigens. Ewing et al. found further that certain strains which possessed related H antigens exhibited a greater or lesser degree of cross-agglutination than did the standard strains. The experiments on phasic variation performed by Vahlne (1945) with ten different H immune sera were unsuccessful.

The relative frequency in various series of *E.coli* strains belonging to the O groups listed in the diagnostic schema is seen in Table 2. According to the results established for the different series a slightly higher percentage of strains from pathological material can be typed than of those from normal material. The percentage of strains belonging to the O groups 1—25 that have been isolated from faeces of healthy adults is seen to vary from 51 to 14 in these series. The majority of the groupable strains have been found to belong to the O groups 1, 2, 4, 6, 8, 15, 17, 18 and 21. O inagglutinable strains have been isolated in higher frequency

from pathological material than from faeces by Kauffmann and coworkers (Kauffmann 1954). In three series of Kauffmann, Knipschildt and Vahlne, such strains amounted to 26, 45 and 65

TABLE 2

RELATIVE FREQUENCY (IN PER CENT) OF STRAINS BELONGING TO O GROUPS 1—25 IN DIFFERENT SERIES

Source	Kauffmann (1944)	Vahlne (1945)	Ewertsen & Knipschildt (1946)	Parvis & Grosso (1953)	Levanto (1954)	Grönroos et al. (1955)	Leppänen (1957)	Wramby (1948)	Fey (1955)
Human									
Pathological									
Peritonitis		73	75	53			88		
Appendicitis ..		78	74	65			76		
Urinary infections	62	61		26		31			
Cholecystitis ..		44							
Suppurative conditions ...				23		33			
Achlorhydric gastric juices .					34				
Normal									
Faeces	14	42	51	24			41		
Appendices ...		68	65				57		
Animal									
Various infections				11				40	40
Normal faeces .				17					
Total no. of examined strains	92	5542	2868	1048	82	483	3500	6011	113

per cent of the strains isolated from faeces. Furthermore, the *Escherichia* flora has been found to be more uniform in pathological specimens than in specimens obtained from normal material (Wramby 1948, Kauffmann 1954 and Leppänen 1957).

According to the investigations of Kauffmann and coworkers (Kauffmann 1954) there is correlation between type, origin,

O inagglutinability, haemolysis, necrotizing action and toxicity. As a rule strains isolated from pathological material are more frequently O inagglutinable, haemolytic, necrotizing and toxic than those isolated from faecal speacimens.

Sears and Brownlee published in 1953 a study on the persistence of individual *E.coli* strains in the intestinal tract of man. The subjects comprised, in addition to three adults, three infants, two of them twins. The twins were followed over a period of one year during which specimens were taken on 45 occasions. The third infant was followed over a period of ten months and specimens were taken on 21 occasions. The twins were two weeks and the third baby ten days old at the time of the first sampling. The authors examined some ten strains isolated from each culture. One-half of these were found to belong to O groups 1, 2, 3, 4, 8 and 17. The authors found that normal individuals harbour some (resident) *E.coli* strains over longer periods and some (transient) over shorter periods.

At the time when the results of this investigation were presented before the Eleventh Scandinavian Congress of Pathology and Bacteriology in Aarhus, no other information had been published about the frequency of *Escherichia* strains of O groups 1—25 in the faeces of infants less than two years old. Ørskov (1956 a, b, c,) also reported to the same congress the results of a similar study of 581 *E.coli* strains isolated from 581 infants suffering from infantile diarrhoea and 183 strains from an equal number of healthy infants. He made use of 130 O immune sera. He found that a large number of the corresponding O groups were represented and that strains of each group were isolated in almost equal frequency from healthy and sick infants.

The significance of *Escherichia* types 26:B6, 55:B5, 86:B7 and 111:B4 as aetiological factors in infantile diarrhoea is now generally recognized (see Kauffmann 1954). These types may be divided into several sub-types according to their H antigens. Some of the sub-types are more frequently encountered during epidemics of infantile diarrhoea than others, which may occur only rarely. *Escherichia* types 125:B15:19 and 126:B16:2 have also been isolated from diarrhoeal infants by Taylor and Charter (1952), but each type in one sporadic outbreak only. Little is known about the world-wide incidence of these two types. Only

low percentages of sporadic cases of infantile diarrhoea have been found by the majority of investigators to be associated with one of the above-mentioned *Escherichia* types, while certain of these types have been isolated in high frequency during epidemics. With regard to the numerous papers on the subject from recent years, reference may be made to previous publications (Braun 1953, Grönroos 1954, Ocklitz 1954, Dupont 1955, Smith 1955 and Ørskov 1956 c).

E.coli strains possessing the O 78 antigen have been rather frequently isolated from calves with white scours (Wramby 1948, Bokhari and Ørskov 1952, Ulbrich 1954, Fey 1955 and Wood 1955). A parallel is often drawn between infantile diarrhoea and diarrhoeal disease of calves; this was the reason for studying also the occurrence of *E.coli* strains of O group 78 in the faeces of healthy and diarrhoeal infants.

PRESENT STUDY

A. MATERIAL

The material of the present investigation was collected during the period from October 1952 to October 1953. During this period 1—4 faecal specimens were taken at intervals of one to three months from 107 healthy infants chosen at random from visitors at three Child Welfare Centres of the City of Turku and from 63 sporadic cases of infantile diarrhoea of which 58 were treated at the Children's Clinic of the University of Turku. Five of the 107 healthy infants became sick with diarrhoea during this study, and were then included in the group of infantile diarrhoea cases.

The age distribution among the 107 healthy and 63 sick infants is shown in Table 3. None of the infants were premature babies.

TABLE 3

AGE DISTRIBUTION OF INFANTS IN MONTHS

Group	0—2	3—6	7—12	> 12	Total number of infants
A. Healthy infants.	25	39	41	2	107
B. Diarrhoeal infants	30	16	10	7	63

In general the sporadic cases of infantile diarrhoea were mild. Marked dehydration was seen in two diarrhoeal infants and severe intoxication following dehydration in three. One of these toxic cases ended fatally. This was the only fatality in the present series.

In all 353 faecal specimens were examined for the presence of *E.coli* types included in the diagnostic Escherichia schema and of *E.coli* types of the following O groups: 26, 44, 55, 78, 86, 111, 125,

and 126. The possible presence of *Shigella* and *Salmonella* in the faecal specimens was also investigated.

As eight coliform colonies were picked from the cultures made from each specimen, the total number of strains examined was 2824.

B. METHODS

BACTERIOLOGICAL TECHNIQUE

Collection of Specimens and Culture Methods. — The stool specimens were either obtained from the diapers or taken by means of rectal swabbings. The specimens were placed in 20-ml vials which contained 4 ml of physiological saline to prevent drying. The rectal swabs were stored inserted in the cork stoppers of the vials. The specimens from the patients of the Childrens Clinic were taken on admission and cultured within a few hours. The specimens from the three Child Welfare Centres of the City of Turku were forwarded to the laboratory after their closing and were cultured on the same day.

The faecal specimens were cultured onto bromo-cresol purple lactose agar and onto either bromo-cresol lactose agar containing 0.1 per cent sodium desoxycholate and 0.1 per cent sodium thiosulphate or SS-agar (Difco). As enrichment medium for the detection of *Salmonella* and *Shigella,* selenite F broth was used.

The cultivation of the specimens was carried out in the same manner as described earlier by the author (1954).

Identification technique. — The actual occurrence of *E.coli* strains was determined by examining the bromo-cresol purple lactose agar plates (berp-plate), while the other media were employed to recover *Salmonella* and *Shigella* strains possibly present.

The existence of *Escherichia* colonies on the berp-plates was studied after overnight incubation at 37°C. Eight coliform colonies from each plate were subcultured twice. Urea decomposition, indole production, and methyl-red reactions of the subcultured strains were first studied. Strains exhibiting urea decomposition were considered not to be *E.coli.* If either of the other two reactions was negative, the following additional tests were performed: fermentation of mannitol (acid and gas) and lactose, hydrogen sulphide production, liquefaction of gelatine, citrate utilization, and Voges-Proskauer reaction. These tests were performed as described previously (Grönroos 1954). When the subcultured strains gave reactions ascribed to *E.coli,* they were studied serologically. All the above-mentioned biochemical tests were carried out also on O groupable strains.

SEROLOGICAL TECHNIQUE

Preparation of Immune Sera. — O, OK and H immune sera were prepared as described in an earlier report (Grönroos 1954). The prepared O immune sera can be seen in Table 4. OK immune sera were prepared for 63 different K antigens, including the 56 belonging to the diagnostic antigenic schema of *E.coli* and 7 belonging to the *E.coli* strains of the other O groups mentioned except O 78. The H antigens were studied using immune sera prepared for H antigens 1—33. The type strains employed in the preparation of the immune sera, with the exception of three, were obtained from Dr. F. Kauffmann and Dr. F. Ørskov of the International Escherichia Centre, Copenhagen. Of the three type strains which were not obtained from the Centre, two strains, E 611 (126:B16) and Canioni (125:B15), were received from Dr. J. Taylor, Central Public Health Laboratory, Colindale, London, and one, D 433, from Prof. G. Olin, State Bacteriological Laboratory, Stockholm.

Specificities of the O Immune Sera. — The specificities of the prepared O immune sera were checked by cross-agglutination tests. The homologous titres of the immune sera were determined using twofold dilutions beginning with the dilution 1 : 50. The homologous titres and overlapping reactions of the O immune sera are shown in Table 4.

The overlapping reactions of the strains belonging to other O groups than 1—25 were reproduced, with one exception, by five separately prepared O immune sera. If we compare the overlapping reactions observed in the cross-agglutination tests in this study with those reported by Vahlne (1945), we see that 34 of the present 64 overlapping reactions occur also in the cross-agglutination schema of Vahlne. The latter schema contains 54 overlapping reactions when only those are counted for which the titres were higher than 1 : 20. Separately prepared O immune sera are found to give significant overlapping reactions fairly regularly. This was emphasized by Vahlne (1945) and Ewing et al. (1956) when they compared the overlapping reactions observed by other workers with their own.

Agglutination Technique. — Since it was obvious beforehand that large quantities of O immune sera would be consumed in the planned work, tests were made to develop a reliable modification which would lower the serum consumption. With this in mind, approximately 1-ml test tubes were used for agglutination tests. In order to determine the reliability of the modification, a com-

Strain	O antigen	Mixed serum A					Mixed serum B				
		1	2	3	10	23	4	13	18	19	25
U 5/41	1	25600	800	—	100	—	—	100	—	—	—
U 9/41	2	400	25600	—	—	—	—	200	—	—	—
U 14/41	3	—	—	25600	100	1600	—	400	—	—	—
Bi 8337/41	10	100	—	—	25600	—	—	—	—	—	—
E 39a	23	—	—	3200	—	12800	—	—	800	—	—
U 4/41	4	—	—	50	—	—	25600	100	800	—	100
Su 4321/41	13	100	—	800	—	—	—	3200	400	1600	100
F 10018/41	18	—	—	100	—	400	1600	200	25600	—	200
F 8188/41	19	—	—	—	—	—	—	100	400	6400	100
E 47/a	25	—	—	—	—	—	200	—	800	400	3200
U 1/41	5	—	—	—	—	—	—	—	—	—	—
Bi 7458/41	6	—	—	—	—	400	—	—	—	—	—
Bi 7509/41	7	—	—	—	—	—	—	—	—	—	—
Bi 626/42	12	—	—	—	—	—	—	—	—	—	—
F 7902/41	15	—	—	—	—	—	800	50	—	—	—
Bi 623/42	11	—	—	—	—	—	—	—	—	—	—
F 11119/41	16	—	—	800	—	—	1600	—	100	—	50
P 7a	20	—	—	—	200	—	—	—	—	—	—
E 19a	21	—	—	—	—	200	—	—	—	—	—
E 41a	24	—	—	—	—	—	—	—	—	—	—
G 3404/41	8	—	—	—	—	—	—	—	—	—	—
Bi 316/42	9	—	—	—	—	—	—	—	—	—	—
Su 4411/41	14	—	—	—	—	—	—	—	—	—	—
K 12a	17	—	—	200	—	—	—	400	—	—	—
E 14a	22	—	—	—	400	100	—	—	—	—	—
	26	—	—	—	—	—	—	—	—	—	—
	44	—	—	—	—	—	—	100	—	—	—
1064	55	—	—	—	—	—	—	—	—	—	—
D 433	111	—	—	—	—	—	—	—	—	—	—
	78	—	—	—	—	—	—	—	—	—	—
E 990	86	—	—	—	—	—	100	100	—	—	—
Canioni	125	—	—	—	—	—	—	—	—	—	—
E 611	126	—	—	—	—	—	—	—	—	—	—

[1] — denotes no agglutination at titre 1:50.

TABLE 4

CROSS-AGGLUTINATION SCHEMA [1]

Mixed serum C					Mixed serum D					Mixed se		
5	6	7	12	15	11	16	20	21	24	8	9	14
200	—	—	—	—	—	—	—	—	—	—	—	—
50	—	—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—	—	—	—
100	100	—	—	—	—	—	—	—	—	—	—	—
—	—	100	—	800	100	200	—	—	—	—	—	—
—	—	—	—	100	—	—	100	—	—	200	—	—
—	—	—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	100	50	—	—	—	—	—
—	100	1600	—	—	—	—	200	—	—	—	—	—
6400	—	1600	—	—	—	—	—	—	—	—	—	—
—	12800	—	—	—	—	—	—	—	—	—	—	—
800	—	25600	—	—	—	—	—	—	—	—	—	—
—	—	—	12800	1600	100	—	—	—	—	—	—	—
—	—	—	—	12800	100	—	—	—	—	—	—	—
—	—	—	—	—	3200	—	—	—	—	—	—	—
—	—	—	—	—	—	25600	—	—	—	—	50	—
—	—	—	—	—	—	100	25600	—	—	—	—	—
—	—	—	—	—	—	—	50	25600	—	—	—	—
—	—	—	—	—	—	—	—	—	400	—	—	—
50	—	50	—	—	—	—	—	—	—	25600	400	—
—	—	—	—	—	—	—	—	—	—	—	3200	—
—	—	—	—	—	—	—	—	—	—	—	—	1600
—	—	—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	100	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—	—	—	—

Mixed serum E				Mixed serum F				Mixed serum G			
	14	17	22	26	44	55	111	78	86	125	126
	—	—	—	—	—	—	—	—	—	—	—
	—	—	—	—	—	—	—	—	—	—	—
	—	—	—	—	—	—	—	—	—	—	—
	—	—	—	—	—	—	—	—	—	—	—
	—	—	—	—	—	—	—	—	100	—	—
	—	—	—	—	—	—	—	—	—	200	—
	—	—	—	1600	50	—	—	—	—	200	—
	—	—	—	—	—	—	—	—	—	—	—
	—	—	—	—	—	—	—	—	800	—	—
	—	—	—	—	—	—	—	—	50	—	—
	—	—	—	—	—	—	—	—	—	—	—
	—	—	—	—	—	—	—	—	—	—	—
	—	—	—	—	—	—	—	—	—	—	—
	—	—	—	—	—	—	—	—	—	200	—
	—	—	—	—	—	—	—	—	—	—	—
	—	—	—	—	—	—	—	—	—	—	—
50	—	—	—	—	—	—	—	—	—	800	—
	—	—	—	—	—	—	—	—	—	—	—
	—	—	—	—	—	—	—	—	—	—	—
	—	—	—	—	—	—	—	—	—	—	—
00	—	—	—	50	—	—	—	—	—	—	—
00	—	—	—	—	—	—	—	—	—	—	—
	1600	—	—	—	—	—	—	—	—	—	—
	—	6400	—	—	—	—	—	—	—	50	—
	—	—	12800	—	—	—	—	—	—	—	—
	—	—	—	12800	—	—	—	—	—	—	—
	—	—	—	—	25600	—	—	—	—	—	—
	—	—	—	—	—	12800	—	—	—	—	—
	—	—	—	—	—	—	12800	—	—	—	—
	—	—	—	—	—	—	—	6400	—	—	—
	—	100	—	—	—	800	—	—	3200	—	—
	—	—	—	—	100	—	—	—	—	6400	—
	—	—	—	—	—	—	—	—	—	—	12800

parison was made of the reactions carried out in these test tubes with those performed in ordinary 10-ml test tubes.

As the storage of the 1-ml test tubes at the optimal reaction temperature of 50°C. in a water bath encountered technical difficulties, the effect of temperature on the agglutination reaction was investigated. Agglutination test series (with approximately 0.20 ml of serum dilution to a tube) in 1-ml test tubes were incubated under different conditions and the titres determined for comparison with the titres of the agglutination reactions of a corresponding series in 10-ml test tubes incubated overnight in a water bath at 50°C. The tests were performed in duplicate. In each series the titres of O immune sera 1—25 were determined.

Comparison of the Results of the O Agglutination Tests in 1-ml and 10-ml Test Tubes. — The results obtained with the 1-ml test tubes and those obtained with the standard method were used to calculate the ratios of the titres. The grand mean of the mean ratios of the agglutination reaction titres for all 25 immune sera carried out under different conditions are given in Table 5.

TABLE 5

COMPARISON OF O AGGLUTINATION TITRES PERFORMED IN 1-ML AND 10-ML TEST TUBES UNDER DIFFERENT CONDITIONS

Incubation conditions	Overnight at room temp.	2 hrs. at 37° and overnight at room temp.	4 hrs. at 37° and overnight at room temp.	Overnight at 37°	2 hrs. at 44° and overnight at room temp.	4 hrs. at 44° and overnight at room temp.	Overnight at 44°
Mean ratio	0.55	1.02	1.16	0.99	1.06	1.02	0.96
S.D.	±0.55	±0.71	±0.66	±0.59	±0.84	±0.73	±0.27
S.E.	±0.11	±0.14	±0.13	±0.12	±0.17	±0.15	±0.05

According to these results, the titres for the series which were kept in the incubators at 37° and 44° C. did not differ. Since space was available in a 37° C. thermostated room, the tubes were subsequently incubated in this room overnight.

Also the effect of the volume of serum dilution taken for the agglutination tests was investigated. Since the 1-ml tubes are very small, Pasteur pipettes were used. Two series were prepared,

one using approximately 0.10-ml volumes and one using approximately 0.20-ml volumes. The titres obtained for these series were compared as above with the titres obtained by the standard procedure. The titre ratios were calculated as described. It was found that the larger volume gave more reliable results, the mean ratio being 0.68 ±0.15 for the 0.1-ml volume and 1.27 ±0.14 for the 0.2-ml volume. In the actual O agglutination screening tests, approximately 0.20-ml volumes were hence employed.

Determination of the O Antigens. — For the preliminary O antigen determinations, pools of O immune sera were used. The first twenty-five O immune sera were combined in groups of five according to the results of the cross-agglutination tests and were designated A to E as shown in Table 4. Two serum pools F and G prepared from the remaining sera comprised four immune sera each (Table 4). Since strains of O groups 8 and 9 may possess thermostable A antigens, a serum pool was prepared from O 8 and O 9 immune sera. The sera were pooled by taking suitable volumes of the immune sera to give a titre of 1:80 for each component serum.

For the tests in the pooled O immune sera as well as in the monovalent O immune sera, broth cultures of the subcultured *E.coli* strains were heated at 100° C. for 2 ½ hrs., or at 120° C. for 2 hrs. for testing in the pooled serum consisting of O 8 and O 9 immune sera. When positive reactions were obtained in one of the pooled sera, further tests were made in the component sera. The pooled sera were tested in three dilutions, 1:80, 1:160 and 1:320, the monovalent sera likewise in three dilutions, 1:160, 1:320 and 1:640. Finally the positive reactions obtained with monovalent sera using 1-ml tubes were confirmed by retesting in 10-ml tubes.

Determination of the K Antigens. — For the determination of the K antigen, the O inagglutinability of the living strain cultured on 0.1 per cent glucose agar was first examined on a slide with diluted (1:2) homologous O immune serum. If the strain did not agglutinate, it was considered to possess a K antigen. If the strain agglutinated, tests were performed in 10-ml tubes with the living and heated strain in O and OK immune sera. When the heated strain agglutinated in the O serum to within one dilution of the full titre and the living strain in titre 1:80 or lower, the strain was considered to possess a K antigen. Each strain with a K antigen was then tested in unabsorbed K immune sera corresponding to the homologous O antigen, first on slides, and when the result was positive, in tubes. If the strain did not agglutinate, it was further tested in all other OK immune sera with the exception of the OA sera, which were used for strains of O groups 8 and 9 only. If the OK immune serum in which the strain under test agglutinated had been prepared with a strain of the same O group, the former strain was re-examined using pure K serum. Unabsorbed OK immune sera were employed when the tested strain belonged to a different O group or

when owing to the lack of a suitable strain the pure K serum could not be prepared. The tube tests were carried out using 0.4 ml volumes. The tubes were incubated two hours in a water bath at 37° C. and then overnight at room temperature.

Determination of the H Antigens. — The motility tests were performed in the same way as described earlier (Grönroos 1954). If no growth of the strain through U-tubes or Craigie tubes was detected even after five passages and if no swarming through the culture medium occurred when the strain was cultured in Craigie tubes for one week at room temperature, the strain was considered non-motile.

For the determination of the H antigen the motile strains was first tested in H immune sera corresponding to the H antigens previously encountered in connection with the O antigen of the strain in question. If the strain did not possess one of the H antigens of the homologous O group, the strain was further investigated in pooled H immune sera.

Seven pooled H sera were prepared by combining four or five monovalent H sera in such ratios as to give a titre of 1:100 for each component serum. These sera were employed in determining H antigens in the same way in principle as in the determination of O antigens. In all H antigen determinations ten-millilitre test tubes were used, the serum dilution volumes being 0.4 ml. Formolized broth cultures were employed and the time of incubation in a 50° C. water bath was two hours.

When an antigen (O, K or H) was agglutinated to within one dilution of the full titre of the serum for its homologous antigen, it was assigned to the corresponding antigen.

C. RESULTS

Of the 2824 strains examined, 2645 exhibited with minor exceptions reactions ascribed to *E.coli*. Strains of O groups 1—25 among the latter numbered 822 (31.1±0.9 %). Seventy-three of these 822 strains were late lactose positive or lactose negative, and a further 30 deviated individually in one or two examined biochemical reactions. Fourteen of the lactose negative strains belonged to O group 1, forty to O group 17 and nineteen to O group 18. The fourteen strains of O group 1 produced no gas from mannitol, were motile, and possessed H antigen 7.

The 822 O groupable strains were found in 176 cultures of different faecal specimens from 99 infants. In addition to strains of O groups 1—25, twenty strains of O group 44 were identified and forty-one strains were diarrhoeal *E.coli* 26:B6 and 55:B5. Thus 33.4±1.0 per cent of the *E.coli* strains could be typed.

Serological properties of E.coli strains. — The antigens determined for all 883 *E.coli* strains isolated from 187 cultures from 119 infants are given in Tables 6 and 7. Three hundred and ninety-

TABLE

THE ANTIGENIC FORMULAE OF THE DIAGNOSED STRAINS

A. Strains conforming with the

B. Strains deviating from the diagnostic

	O 1				O 2				O 3			
	K and H antigens	No. of strains	No. of cultures	No. of infants	K and H antigens	No. of strains	No. of cultures	No. of infants	K and H antigens	No. of strains	No. of cultures	No. of infants
	Total	126			Total	97			Total	36		
A	1L:7	88	23	21	1L:6	2	1	1	2a, bL:2	8	1	1
	51L:7	19	6	6	1L:7	1	1	1				
	1L: —	9	3	3	1L: —	20	6	5				
					2a, bL:1	4	1	1				
					5L:4	22	4	4				
					5L: —	11	2	2				
B	12L:7	1	1	1	13L:1	9	3	3	?K:2	22	6	6
	21L: —	1	1	1	15L:1	24	5	5	?K:?	6	2	2
	?K:1	1	1	1	20L:1	1	1	1				
	?K: —	1	1	1	45K:1	2	1	1				
	— :7	6	3	3	R: —	1	1	1				

	O 7				O 8				O 9			
	Total	33			Total	30			Total	22		
A	1L: —	29	5	5	8L:20	1	1	1	36K:—	1	1	1
					25B: 9	5	1	1				
B	4L:6	2	1	1	8L:?	1	1	1	?K:10	15	6	6
	5L:1	2	1	1	14L:9	1	1	1	— :12	1	1	1
					25B:?	9	2	2	— : ?	5	1	1
					43K: —	7	2	2				
					46K:2	1	1	1				
					46K:?	2	1	1				
					?K:14	2	1	1				
					?K: —	1	1	1				

	O 13				O 14				O 15			
	Total	8			Total	1			Total	19		
A												
B	?K:11	3	1	1	—:4	1	1	1	13L:—	1	1	1
	— :11	3	1	1					16L,18L:25	4	2	2
	— : ?	1	1	1					18L:12	8	1	1
	— :5	1	1	1					20L:11	3	1	1
									20L:20	1	1	1
									20L:—	2	2	2

	O 19				O 21				O 22			
	Total	3			Total	11			Total	7		
A					20L: —	3	1	1	13L: 1	3	1	1
B	5L: —	1	1	1	20L:12	4	1	1	13L:—	4	1	1
	?K: —	2	1	1	20L:25	3	1	1				
					?K: 4	1	1	1				

ISOLATED FROM FAECES OF HEALTHY INFANTS

diagnostic schema (O 44 included)

schema in respect of K and H antigens

O 4				O 5				O 6			
K and H antigens	No. of strains	No. of cultures	No. of infants	K and H antigens	No. of strains	No. of cultures	No. of infants	K and H antigens	No. of strains	No. of cultures	No. of infants
Total	86			Total	4			Total	65		
3L:5	6	2	2					13L:1	9	3	3
12L:5	44	12	7					53K: —	1	1	1
?K:5	12	3	3	— :10	4	1	1	1L:31	1	1	1
— :4	1	1	1					2a, bL: —	1	1	1
— :5	22	10	10					2a, bL: ?	2	1	1
								5L:1	3	2	2
— :?	1	1	1					5L: —	10	3	3
								53K:1	7	1	1
								?K:1	10	3	3
								?K:31	8	2	2
								— :1	3	3	3
								— :10	3	1	1
								— :33	7	2	2

O 10				O 11				O 12			
Total	33			Total	3			Total	9		
5L:4	20	4	4								
2L: —	1	1	1	52K:4	3	3	3	14L:4	1	1	1
5L: —	11	4	4					24L:?	6	1	1
—: —	1	1	1					?K: —	2	1	1

O 16				O 17				O 18			
Total	3			Total	59			Total	38		
								5L: —	22	6	6
?K: —	3	1	1	16L:11	8	1	1	52K:5	4	1	1
				16L: —	11	3	3	?K:14	6	2	2
				5L: —	18	6	6	?K:16	4	1	1
				R: —	22	3	3	— :14	2	1	1

O 23				O 44			
Total	10			Total	20		
18L:15	7	1	1	44:74L:—	20	4	4
18L: —	3	2	2				

Total No. of
Strains 723
Cultures 153
Infants 89

TABLE 7

THE ANTIGENIC FORMULAE OF THE DIAGNOSED STRAINS ISOLATED FROM FAECES OF INFANTS WITH INFANTILE DIARRHOEA

A. Strains conforming with the diagnostic schema (26:6B and 55:5B included).
B. Strains deviating from the diagnostic schema in respect of K and H antigens.

	O 1				O 2				O 4				O 6			
	K and H antigens	No. of strains	No. of cultures	No. of infants	K and H antigens	No. of strains	No. of cultures	No. of infants	K and H antigens	No. of strains	No. of cultures	No. of infants	K and H antigens	No. of strains	No. of cultures	No. of infants
	Total	24			Total	8			Total	8			Total	4		
A	1L: 7	20	6	6	5L: 4	8	1	1	12L: 5	7	2	2	13L: 1	4	1	1
	1L: —	2	1	1												
B	10L:10	2	1	1					?K: 5	1	1	1				

	O 7				O 8				O 9				O 10			
	Total	6			Total	17			Total	5			Total	2		
A	1L: —	6	1	1	8L:20	1	1	1								
					49K:21	5	1	1								
B					43K: —	3	1	1	?K: ?	5	2	2	1L: 7	2	1	1
					?K: 8	7	1	1								
					?K:33	1	1	1								

	O 13				O 15				O 16				O 17			
	Total	4			Total	13			Total	6			Total	3		
A	5L: 7	3	1	1	20L: —	13	3	3	20L:—	6	1	1	16L: —	2	1	1
B	?K: —	1	1	1									?K: —	1	1	1

	O 21				O 22				Diarrhoeal E.coli type				Total No. of
	Total	14			Total	5			Total	41			Strains 160
A					13L: 1	5	1	1	26:6B:11	11	2	2	Cultures 34
									55:5B:—	9	2	2	Infants 30
B	?K: —	14	3	2					55:5B: 4	8	1	1	
									55:5B: 6	13	2	1	

three (47.8±1.8 %) of the 822 strains of O groups 1—25 were found to conform with the diagnostic antigenic schema. The remaining 429, which thus deviated in respect of their K and H antigens, included strains which may have lost their K antigens as well as strains which could not be changed into actively

motile forms and were hence considered non-motile. The strains belonging to the same O group in the same faecal culture were in about half of the cultures found to possess different K and H antigens, and hence were not identical serologically. On the basis of the data summarized in Tables 6 and 7, strains exhibiting no K antigen comprised 8.5±1.0 per cent of strains isolated from healthy infants while no such strains were isolated from the diarrhoeal infants. The K antigen could be determined in 84.3±1.4 per cent of the O inagglutinable strains. Among all the 883 *E.coli* strains 606 strains were found motile. The H antigens of 574 motile strains (94.7±0.9 %) could be determined.

Antigenic Relationships. — In the majority of the O groupable strains, the homologous O titres varied from 1:5120 to 1:20480. As judged by the heterologous O titres, the relationships between the strains were considered moderate when the titres were 1:80—1:320 and strong when the titres were 1:640—1:2560. Strains belonging to O groups 1, 2, 4, 17 and 18 gave strong overlapping reactions with more than three O immune sera. No heterologous reactions with O titres higher than 1 : 40 were exhibited by strains of O groups 5, 6, 8, 9, 11, 14, and 16. Strains giving overlapping reactions are grouped according to O group and heterologous O titre in Table 8.

Only a few heterologous reactions were encountered in connection with the K antigen determinations for strains whose K antigens did not conform with K antigens given in the diagnostic antigenic schema for the O group in question.

As motile strains were first tested in H immune sera corresponding to the H antigens of the O groups of the strains under test, H antigen relationships were studied only for strains whose H antigens were examined using pooled H immune sera. Heterologous titres were obtained of strains possessing H 1 antigen in H 12 and 16 immune sera. Two *E.coli* strains of sero-type 7:5L:1 were found to give overlapping reactions in H immune sera 2, 4 and 6 with 1:640 titres. Strains with H antigen 12 gave strong overlapping reactions in the H 1 immune serum and strains possessing H 4 antigen in H 17 immune serum. Heterologous reactions were also produced in immune sera H 21 and H 15 by H 11 strains and in H 1 and H 7 immune sera by one H 31 strain each.

TABLE 8

INTER-O GROUP RELATIONSHIPS

The O antigen numbers are given in bold-face type and numbers of strains in ordinary type.

O antigen	Total no. of strains	Strains giving overlapping	Reactions in heterologous immune sera	
			Titres 1:640—1:2560	Titres 1:80—1:320
1	150	86	**2** (11), **3** (1), **10** (38), **13** (18), **18** (3)	**2** (11), **3** (2), **10** (4), **13** (4), **18** (4), **25** (1)
2	105	53	**1** (10), **3** (1), **13** (37), **18** (18), **19** (10)	**1** (1)
3	36	3	**10** (1), **23** (1), **25** (1)	**10** (1)
4	94	79	**7** (29), **12** (13), **13** (43), **15** (17), **16** (3), **18** (17), **19** (5), **25** (7)	**12** (2), **13** (10), **16** (2), **18** (23), **19** (13), **25** (19)
7	39	21	**25** (17)	**5** (4)
10	35	16	**1** (1), **3** (16)	**1** (1)
12	9	9	**15** (7)	**15** (2)
13	8	8	**1** (1), **19** (8), **25** (2)	**18** (2), **25** (1)
15	32	21	**12** (7), **16** (20)	**12** (5)
17	62	42	**4** (3), **13** (3), **18** (23), **19** (6), **23** (31), **25** (13)	**4** (1), **18** (7), **19** (1), **23** (1), **25** (1)
18	38	27	**4** (5), **13** (12), **17** (4), **19** (5), **23** (7), **25** (11)	**4** (6), **7** (2), **19** (1), **25** (1)
19	3	1	**2** (1)	**4** (1)
21	25	4	**24** (4)	
22	12	4		**23** (4)
23	10	7	**3** (5)	**3** (2)

Occurrence in Faeces of Healthy and Diarrhoeal Infants. — None of the faecal specimens examined was found positive for *Shigella,* but *Salmonella paratyphi B* was isolated from a six-month-old diarrhoeal patient. None of the seven *E.coli* strains isolated from the specimens positive for the *Salmonella* type could be typed with the immune sera used in the present study.

The forty-one diarrhoeal *E.coli* sero-types comprised 8.8±1.3 per cent of the 498 *E.coli* strains isolated from 79 faecal specimens obtained from 63 sporadic cases of infantile diarrhoea. The forty-one strains were from 6 diarrhoeal infants; *E.coli* 55:B5:6 and 55:B5:4 were each isolated from one infant, and *E.coli* 55:B5:— and 26:B6:11 each from two infants.

In the evaluation of the part played by *E.coli* strains of O groups 1—25 in infantile diarrhoea, the case found positive for *Salmonella paratyphi B* and the 6 cases found positive for *E.coli* 55:B5 and 26:B6 have been omitted from the group of diarrhoeal infants. Consequently the number of diarrhoeal infants reduces to 56, the number of cultures to 68, and the number of strains to 407.

Eighty-nine or about four-fifths of the 107 healthy infants were found positive for one or several of the O groups 1—25, while twenty-three or less than one half of the diarrhoeal infants were found positive. Table 9 shows that strains of O groups 1—25

TABLE 9

STRAINS GROUPABLE AND NONGROUPABLE WITH O IMMUNE SERA 1—25

	Number of strains from healthy infants	%	Number of strains from diarrhoeal infants	%	Total	%
Number of groupable strains	703	32.9±1.0	119	29.2±2.3	822	32.2±0.9
Number of non-groupable strains	1444	67.1±1.0	288	70.8±2.3	1732	67.8±0.9
Total	2147	100.0	407	100.0	2554	100.0
Number of cultures	274		68		342	
Number of infants	107		56		163	

were isolated in almost equal frequancy from the healthy infants (32.9±1.0 %) and from the infants with diarrhoea (29.2±2.3 %).

The distribution of the identified strains according to O antigen in the groups of healthy and sick infants is seen in Figure 1. The majority (68.5±1.6 %) of the O 1—25 groupable strains belonged to the O groups 1, 2, 4, 6, 8, 17 and 18. It is further seen that 0.9 per cent of the 2147 *E.coli* strains from the healthy infants were of type 44:74L. No sero-types previously

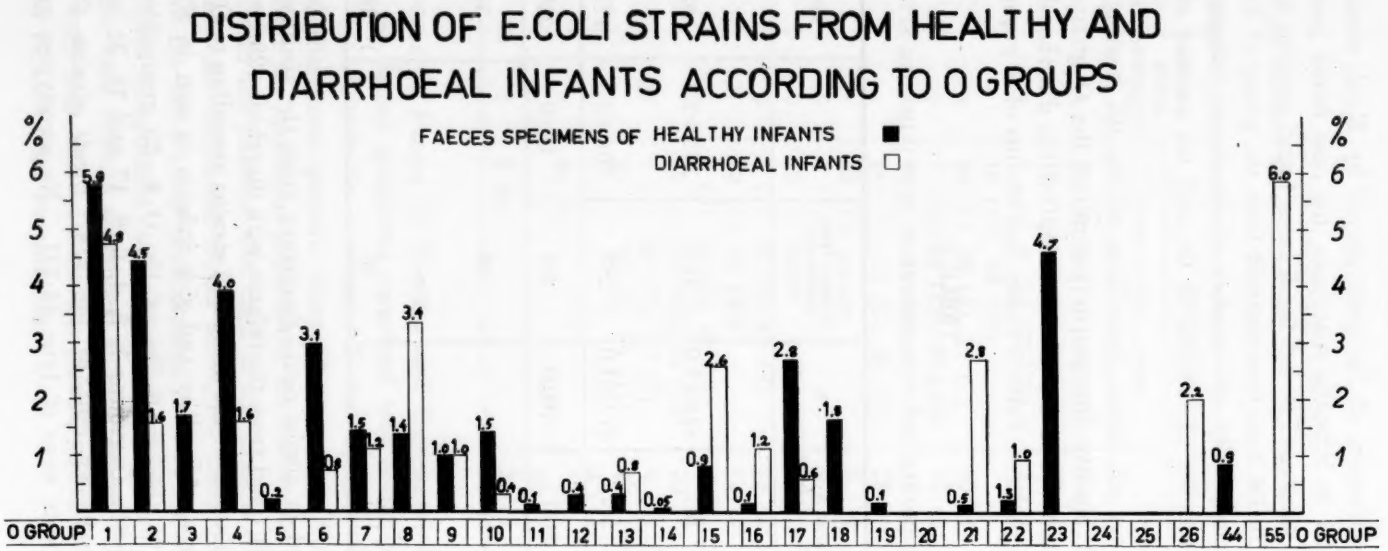
DISTRIBUTION OF E.COLI STRAINS FROM HEALTHY AND DIARRHOEAL INFANTS ACCORDING TO O GROUPS
FAECES SPECIMENS OF HEALTHY INFANTS
DIARRHOEAL INFANTS
%
6
5
4
3
2
1
5.9
4.9
4.5
1.6
1.7
4.0
1.6
0.2
3.1
0.8
1.5
1.2
1.4
3.4
1.0
1.0
1.5
0.4
0.1
0.4
0.4
0.8
0.05
0.9
2.6
0.1
1.2
2.8
0.6
1.8
0.1
0.5
2.8
1.3
1.0
4.7
2.1
0.9
6.0
O GROUP
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
44
55
O GROUP

Figure 1

found associated with infantile diarrhoea were found in this group of healthy infants. The diarrhoeal *E.coli* sero-types 55:B5 and 26:B6 were isolated from cases of infantile diarrhoea only. None of the isolated *E.coli* strains was found to possess the O antigen 78.

As it has been established that the *Escherichia* strains isolated from pathological processes are generally more uniform than those isolated from normal material, it was of interest to determine whether the faecal specimens of healthy and diarrhoeal infants differ in this respect. Table 10 shows the number of cultures from

TABLE 10

DISTRIBUTION OF CULTURES CONTAINING STRAINS OF ONE OR SEVERAL O GROUPS

	Healthy infants		Diarrhoeal infants	
	No. of cultures	%	No. of cultures	%
Cultures from which the isolated strains were:				
a) of the same O group	22	8.1±1.7	11	13.7±3.9
b) of two or more O groups ..	26	9.4±1.8	1	1.3±1.8
c) groupable and ungroupable	105	38.4±3.0	21	26.3±4.3
d) ungroupable	121	44.1±3.0	47	58.7±5.9
Total	274	100.0	80	100.0
Cultures from which more than half of the isolated strains belonged to the same O group	85	31.0±2.8	26	32.5±5.2

which strains belonging to one or several O groups were identified. In only thirty-three cultures from healthy and sick infants did all the identified strains belong to the same O group. These cultures amount to 17.6±2.8 per cent of the 187 which contained O groupable strains. The determination of K and H antigens revealed, however, that in sixteen cultures (8.6±2.0 %) all the identified strains were of the same sero-type. On the other hand, cultures in which at least half of the strains were of the same O group amounted to about 30 per cent in both groups of infants.

The same *E.coli* sero-types were isolated from faecal specimens taken on two different occasions in the case of ten infants and from specimens taken on three different occasions in the case of three infants.

DISCUSSION

Whereas the occurrence of *E.coli* strains belonging to the O groups 1—25 has been studied in adults by numerous investigators (Kauffmann 1944, Vahlne 1945, Knipschildt 1945, Ewertsen and Knipschildt, Parvis and Grosso 1953, Levanto 1954, Grönroos et al. 1955 and Leppänen 1957), the occurrence of these O groups was examined in the present study in faecal specimens from healthy and diarrhoeal infants.

Most of the 822 O groupable *E.coli* strains (68.5±1.6 %) belonged to the groups 1, 2, 4, 6, 8, 17 and 18. This finding conforms with the results of earlier investigators. The percentages of O inagglutinable strains found in the present series is high (91.5±1.0 %) compared with the percentages varing from 26 to 65 per cent reported by Kauffmann and coworkers (1954). This was to be excepted as 68.5±1.6 per cent of the O groupable strains belonged to groups known to contain mainly O inagglutinable strains.

The determination of K and H antigens made in the present study revealed that the *E.coli* strains of the same faecal culture differed in their antigenic structure in about half of the cultures. Thus an *Escherichia* flora that is uniform as judged by its O antigen may often include strains which differ in their K and H antigens. The same is indicated by Ørskov's (1956 b, c) observation that strains belonging to the same O group may differ in their biochemical behaviour. In 8.6±2.0 per cent of cultures which contained O groupable strains in the present series, all the strains were found to be of the same sero-type. In the case of thirteen infants, the same sero-type was cultured from faecal specimens taken on two or three different occasions. It is therefore possible that the conclusions of Sears et al. (1950, 1952) relating to the occurrence of resident and transient *E.coli* strains in the intestinal tract of man might have been different had they determined also the K and H antigens of the strains they isolated.

With respect to major antigenic interrelationships between type strains and between isolated strains found when determining antigens, there were only few deviations when compared with the interrelationships observed by other workers. It is likely that strains possessing a certain antigen can also possess a variety of partial antigens. Thus strains are inadequately defined by determining only O antigens without performing cross-absorption tests. A more exact serological definition is reached by determining also K and H antigens. The reasons for this are the few overlapping reactions encountered with these antigens and the fact that the H antigens of almost all motile strains can be determined.

No significant difference was noted between the relative numbers of O 1—25 groupable strains isolated from healthy and from diarrhoeal infants. On the other hand, 8.8±1.3 per cent of the *E.coli* strains isolated from faeces of diarrhoeal infants were of the diarrhoeal *Escherichia* sero-types 55:B5 and 26:B6, but these types were not found among those isolated from the non-diarrhoeal infants, who, being healthy visitors at Welfare Centres, can be considered non-contacts.

Whereas diarrhoeal *E.coli* strains are frequently isolated in pure culture from faecal specimens of diarrhoeal infants, cultures containing strains of O groups 1—25 were not found to be more uniform in cultures of faeces from diarrhoeal infants than in those from healthy infants. Cultures in which at least half of the strains were of the same O group amounted to about 30 per cent in both groups of subjects. Even this degree of uniformity is illusory since, as mentioned above, more detailed serological identification revealed heterogenity within the O groups of the same culture. This is in agreement with the conclusion of Ørskov (1956 b) despite the fact that he strove to select uniform colonies on the basis of closely similar morphological appearance.

Since Ørskov's material (Ørskov 1956 b, c) is very similar to the present material, it is of interest to compare the incidence of strains of the most common of the groups 1—25 in the two series (Table 11). The relative frequencies of strains of several O groups are almost equal in both series. Ørskov's series is seen to contain a slightly greater proportion of O 1—25 groupable strains than the present series. This may be due to the fact that Ørskov classified a strain as belonging to an O

TABLE 11

DISTRIBUTION (%) OF THE EXAMINED E.COLI STRAINS INTO THE MOST COMMON OF O GROUPS 1—25 IN ØRSKOV'S (1956) MATERIAL (I) AND IN THE PRESENT SERIES (II)

O group	Infants with diarrhoea		Healthy infants	
	I	II	I	II
1	4.0	5.9	5.5	5.9
2	7.2	1.9	12.6	4.5
3	0.9	—	0.5	1.7
4	4.0	1.9	6.6	4.0
6	5.3	0.9	6.6	3.1
7	—	1.5	—	1.5
8	6.9	4.2	2.7	1.4
9	1.5	1.2	1.1	1.0
10	—	0.5	—	1.5
12	1.0	—	0.5	0.4
15	2.9	3.2	0.5	0.9
17	—	0.7	—	2.8
18	1.0	—	1.6	1.8
21	3.6	3.4	2.2	0.5
Total	38.3±2.1	25.3±2.6	40.4±3.7	31.0±1.0
Strains examined	581	407	183	2147

group if the strain gave a titre of 640 or more in the O serum concerned, while in the present study a strain was considered to possess an O antigen if the strain was agglutinated by an O immune serum in a dilution that was the same as or deviated not more than one dilution from that for the type strain. The incidence in the present series, like the incidence in Ørskov's series, was so low for all O groups that no conclusion can be drawn about the relationship of these groups to infantile diarrhoea.

SUMMARY

The serological identification of *E.coli* O groups 1—25, 44 and 78 and sero-types 26:B6, 55:B5, 86:B7, 111:B4, 125:B15 and 126:B16 and their possible occurrence in faeces of healthy and diarrhoeal infants under two years of age has been investigated.

A total of 2147 *E.coli* strains have been isolated from 274 faecal specimens of 107 healthy infants and 498 from 79 faecal specimens of 63 diarrhoeal infants. The serological properties of these strains have been studied using immune sera prescribed in the diagnostic antigenic schema of *E.coli* (according to Kauffmann, Knipschildt and Vahlne), immune serum O 78, O and K immune sera for *E.coli* types 26:B6, 44:74L, 55:B5, 86:B7, 111:B4, 125:B15 and 126:B16, and immune sera H 1—33.

The O antigens of 33.4±1.0 per cent of the isolated *E.coli* strains could be determined. 47.8±1.8 per cent of strains belonging to O groups 1—25 were found to conform with the diagnostic antigenic schema of the *Escherichia coli*. 68.5±1.6 per cent of all O 1—25 groupable strains belonged to the most common groups 1, 2, 4, 6, 8, 17 and 18. Strains belonging to O groups 1, 2, 4, 17 and 18 gave overlapping reactions with more than three heterologous O antisera. No overlapping reactions in heterologous O immune sera with titres higher than 1:40 were exhibited by strains of O groups 5, 6, 8, 9, 11, 14 and 16.

Strains exhibiting no K antigen comprised 8.5±1.0 per cent of strains isolated from healthy infants while no such strains were isolated from the diarrhoeal infants. The K antigen could be determined for 84.3±1.4 per cent of the O inagglutinable strains.

The H antigens could be determined for 94.7±0.9 per cent of all motile strains.

Determinations of K and H antigens revealed that the *E.coli* strains of the same O group isolated from the same faecal culture may differ in their antigenic structure. Only in 8.6±2.0 per cent

of cultures containing O groupable strains were all strains found to possess a similar antigenic structure.

Of the strains isolated from the healthy infants 32.9±1.0 per cent were groupable with immune sera O 1—25, while the percentage for the diarrhoeal infants was 29.2±2.3. No significant differences were hence noted in the occurrence of the O groups in the two groups of subjects.

Twenty *Escherichia* 44:74L:— strains were isolated from 4 healthy infants, but none from the diarrhoeal infants, whereas diarrhoeal *E.coli* sero-types were isolated only from the latter group of infants as follows: *E.coli* 55:B5:6 and 55:B5:4 were each isolated from one infant, and 55:B5:— and 25:B6:11 each from two infants.

None of the strains examined was found to belong to O group 78, which has been frequently isolated from calves with white scours.

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J. A. Grönroos

Department of Medical Microbiology, University of Turku,
Kiinamyllynk. 8,
Turku, Finland.

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